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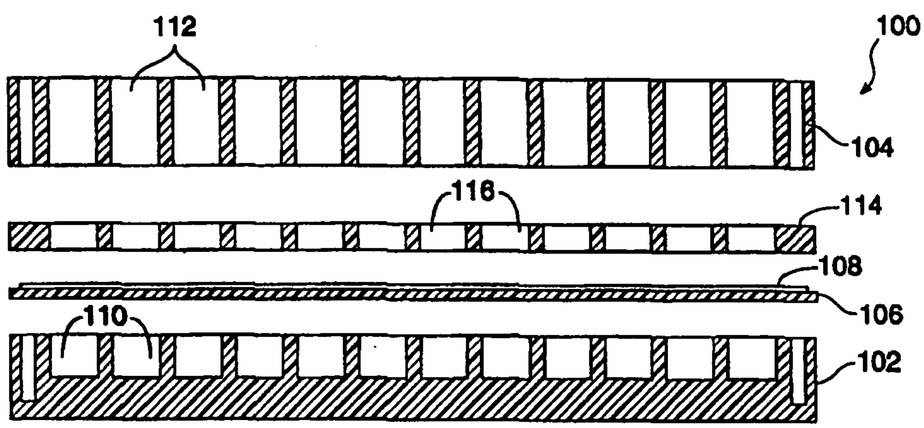

(57) Abstract

The invention provides exemplary testing devices, systems and methods for evaluating the permeation of various chemicals through different types of cells. In one exemplary embodiment, a testing device (100) is provided which comprises a base member (102) and a top member (104) having a plurality of wells (110) which are aligned when the top member (104) is secured to the base member (102). A membrane sheet (106) which includes at least one layer of cells grown on the sheet (106) is placed between the base member (102) and the top member (104) prior to assembly. Test samples are placed into the wells (110) in the top member (104) and samples are removed from the top and bottom wells (110) at a later time and tested to determine the amount of test sample which permeated through the cells.

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MULTI-WELL SINGLE-MEMBRANE PERMEATION DEVICE AND METHODS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation in part application of U.S. Patent Application Serial No. 08/959,434, filed October 28, 1997, the complete disclosure of which is herein incorporated by reference.

BACKGROUND OF THE INVENTION

This invention relates generally to the field of testing systems and methods, and more particularly to systems and methods for transport or permeation testing. In one particular aspect, the invention provides systems and methods for culturing cells onto a membrane and then using the membrane to simulate an epithelial cell layer, such as the cells which form the inner lining of a human intestine, the blood-brain barrier or blood vessels. In this way, transport or permeation tests may be performed using the membrane.

In humans, ingested food passes from the stomach to the small intestine where proteins, fats, carbohydrates and other nutrients are absorbed and distributed into circulation for use in various organs and cells throughout the body. The small intestine is about five to six meters in length and has an extremely large surface area for absorbing nutrients and other materials. The interior of the small intestine includes the mucosal epithelium which comprises small fingerlike projections called villi which protrude into the intestinal lumen and provide the nutrient absorption surface.

For a variety of reasons, it is desirable to study and evaluate how various drugs and other chemicals which are orally ingested by a human will be absorbed into the blood stream through the intestinal wall. Such evaluation can be useful in, for example, drug testing to determine how various

drugs would permeate through the intestinal wall and be absorbed into the blood stream after being orally ingested. Determining transport of various substances through other types of epithelial cells can also be useful in therapeutically treating patients.

In order to evaluate how certain chemicals or other substances will permeate epithelial cells, some have proposed growing mammalian-based cells on a membrane which in turn is used to mimic a cell layer within the body. Some previously proposed testing systems comprise a cup having a membrane at its bottom end. After the cells have grown onto the membrane, the cup is inserted into a larger cup or well and various chemicals are placed into the upper cup to evaluate how the chemicals will permeate the cells on the membrane and enter into fluid in the bottom well.

Such testing systems suffer from a variety of drawbacks, including the significant amount of time required to separately seed the cells into each of the upper cups and to add and replace cell culture nutrients in each cup at regular intervals. A further drawback to such systems is their limited use in accommodating smaller sized membranes. For example, many multi-well plates are being provided with increased numbers of wells whose dimensions are significantly smaller to create larger densities of wells within the plates. Accordingly, each upper cup and its membrane needs to be made smaller in order to fit within the smaller wells. However, when reducing the size of the membranes with the testing systems described above, the membrane's surface area may be too small to provide an adequate transport interface. In turn, this can lower concentrations or transported amounts to levels which restrict analytical methodologies presently available to quantify results. Further, the activity provided by a cell layer on such small membrane sizes may not be representative of the activity provided by a cell layer on a larger membrane.

Hence, for these and other reasons, it would be desirable to provide systems and methods which will allow cells to be seeded horizontally in an efficient manner. In

some cases it would also be desirable to allow access to both sides of the membrane during a testing procedure. Further, it would be desirable to provide a design for a testing system where membrane densities are greatly increased while still being sufficiently sized to effectively accommodate cell growth and to provide an adequate transport interface.

SUMMARY OF THE INVENTION

The invention provides systems, devices and methods that are useful in performing various transport or permeation tests, preferably in order to simulate the transport of various substances through epithelial cell layer, such as the cells which line the human intestine, blood vessels, and the like. In one exemplary embodiment, the invention provides a testing device which comprises a plate defining at least one well. At least one membrane is provided which is insertable into the well to divide the well into separate chambers. The membrane is removable from the well to allow cells to be grown on the membrane before insertion into the well. With this arrangement, the membrane may be horizontally oriented to allow cells to be seeded on the membrane. Subsequently, the membrane may be inserted into the well and permeation tests performed.

In one exemplary aspect, the device further includes a divider to which the membrane is operably attached. The divider is insertable into the well to hold the membrane in a generally vertical orientation within the well. In this way, the transport interface between the chambers is maximized to in turn maximize transport between the chambers. Although a generally vertical orientation is preferred, it will be appreciated that in some cases the membrane may be inclined or even horizontal.

Preferably, the divider is arranged so that it encompasses a periphery of the membrane. Further, the divider will preferably form a raised edge around the periphery of the membrane to constrain cell growth to the membrane. In one particular aspect, the divider comprises a half-well having a planar face to which the membrane is operably attached. In

this manner, the half-well may be inserted into the well to divide the well into separate chambers.

In still a further aspect, the plate defines a plurality of wells, and a plurality of membranes are provided which are insertable into the wells in a generally vertical orientation. The vertical arrangement of the membranes in this manner is advantageous in that large numbers of wells may be provided within a single plate, with each membrane being sufficiently sized so that the cells may be properly cultured thereon prior to insertion.

In one particular aspect, the divider comprises a plurality of teeth to which the membranes are operably attached. A bridge is further provided to interconnect the teeth. In this way, the teeth may be inserted into a row of wells to place a separate membrane into each well. In an alternative aspect, the divider comprises an elongate member to which the membranes are operably attached. With this arrangement, the plate preferably includes an elongate slot extending between the array of wells. In this way, the elongate member may be inserted into the slot to place the membranes into the wells. In still a further alternative aspect, the divider comprises a plurality of half-wells, each having a planar face to which the membranes are operably attached. The half-wells are insertable into the wells to place the membranes into the wells. Preferably, the half-wells will be operably attached to each other to create a strip of half-wells.

In still a further aspect, the wells are arranged in a two-dimensional array of rows and columns. With this configuration, a plurality of row dividers are provided, each having a plurality of membranes operably attached thereto. In this way, each row may have its wells divided by inserting the dividers into the rows. In another aspect, the membrane is constructed from materials such as polytetrafluroethylene, polyethylene, PET, polycarbonate and the like.

In another exemplary embodiment, the invention provides a testing system which comprises at least one membrane and a cell culture device having cells therein. In

this way, the membrane may be placed into the cell culture device in a horizontal orientation to allow the cells to be seeded on the membrane. The system further includes a plate having at least one test well into which the membrane may be inserted after cells have been grown on the membrane. In this way, the membrane divides the well into separate chambers so that various tests may be performed.

In one particular aspect, the cells within the cell culture device comprise mammalian-based cells. In another aspect, the system further includes a means for measuring the concentration of a substance within each chamber after the substance has been placed into one of the chambers and has diffused into the other chamber. In this way, the permeation rate of the substance through the membrane may be determined. In still another aspect, a means is provided for introducing the substance into the well after placement of the membrane into the well. The system may further include a variety of dividers as described above which are insertable into the well to hold the membrane in a generally vertical or inclined orientation within the well.

In one exemplary embodiment, the invention provides a testing device which comprises a base member having a plurality of wells. A top member is also provided having a plurality of apertures which correspond to the wells of the base member. A membrane sheet is further provided to receive a layer of cells and to be placed between the top member and the base member, forming top and bottom wells. In this way, a substance that is placed within the top wells may permeate through the membrane and into the bottom wells. The membrane is removable from the device to allow cells to be grown on the membrane before insertion into the device. Such a system is therefore advantageous in that the cells may be seeded and grown on the membrane in an appropriate environment. The membrane sheet may then be placed between the base member and the top member to facilitate a testing procedure. Such a system is particularly advantageous in that only one membrane sheet is seeded with cells and cultured for each multiple-well device. In this way, testing of substances is made more

efficient because multiple wells share the same membrane which is easily placed between the top and base members.

In a further aspect, the wells are arranged in a two-dimensional array of rows and columns. Preferably, the arrangement of these rows and columns is chosen to be compatible with multi-well pipettes or automated liquid handling systems to further facilitate testing procedures. Optionally, a gasket may be provided and be placed between the base member and the membrane or between the top member and the membrane to provide a seal between the membrane and base member or the top member. In another aspect, a securing mechanism may be provided to secure the top member to the base member.

In another aspect, the membrane is constructed from materials such as polytetrafluroethylene, polyethylene, PET, polycarbonate and the like. Further, to facilitate growth of the cells the surface area of the membrane may be framed to constrain growth of the cells onto the membrane and to provide rigidity to the membrane. As one example, the frame may comprise a ring or cup-shaped (or other geometrically shaped) member which is disposed about a periphery of the membrane. In one particular aspect, the cells seeded and cultured on the membrane comprise mammalian-based cells.

In a still further aspect, the base member is constructed with a partial wall whose shape is complementary to the shape of the membrane and its frame. This wall acts as a guide to ensure reliable and reproducible insertion of the membrane into the device without damaging any cells on the membrane. One particular advantage of using a cup-shaped frame is that the top member may be held within the frame, with the frame (in combination with the guide of the base member) serving to align the apertures of the top member with the wells of the base member. In another aspect, one ore more pins protrude from a bottom face of the top member and correspond to holes in a top face of the base member. The pins and corresponding holes assist in the alignment of the apertures in the top member with the wells in the base member, and prevent rotational stress on the layers of cells.

In one particular embodiment, the apertures in the top member have a larger cross-section than the wells of the base member. This may be accomplished, for example, by bevelling a bottom edge of the apertures in the top member. In this way, the top member does not crush the cells near the edge of the wells in the base member when the top member is forced against the base member to make a seal.

In one particular aspect, multiple base members may be provided with different sized wells. In this way, different base members may be employed to control the flow rate of the substance through the membrane.

In another aspect, the system further includes a means for measuring the concentration or other characteristic of a substance within each chamber after the substance has been placed into one of the chambers and has diffused into the other chamber. In this way, the permeation rate of the substance through the membrane and cell layer may be determined. To facilitate evaluation of the characteristic, samples from the lower well may be obtained either by removing the membrane sheet from the base layer or by puncturing the membrane sheet with a sampling device.

In a preferable aspect, the evaluated characteristic is the concentration of the substance. In this way, the permeation rate of the substance through the cells on the membrane may be determined based at least in part on the measured concentrations of the substance within the top and bottom wells after a given period of time. To facilitate evaluation, the substance preferably comprises a chemical which is contained in a buffer which is identical or similar to the buffer in the top and bottom wells.

In one optional aspect, the wells may be filled with a buffer solution prior to placement of the membrane sheet between the top member and the base member. In another aspect, the substance may be maintained within a desired temperature range while permeating the membrane sheet. One particularly advantageous feature of the method is that the membrane sheet may be either horizontally oriented or vertically oriented during permeation.

The invention still further provides an exemplary method for performing assays. According to the method, cells are grown onto a membrane. The membrane is then inserted into a well to divide the well into a donor chamber and a receptor chamber. At least one substance is then introduced into the donor chamber and is allowed to diffuse through the membrane into the receptor chamber. A characteristic of the substance that is within the donor chamber and the receptor chamber is then evaluated over time.

In one particularly preferable aspect, the cells are seeded onto the membrane while the membrane is in a generally horizontal orientation. Such a horizontal orientation is particularly useful when the cells comprise mammalian-based cells. Further, to facilitate growth of the cells the surface area of the membrane may be framed to constrain growth of the cells onto the membrane.

In another particular aspect, the membrane is operably attached to a divider and the divider is inserted into the well in a generally vertical orientation. In one aspect, the well includes at least one generally vertically oriented slot into which the divider is slid to insert the divider into the well. In an alternative aspect, the divider comprises a half-well having a planar face to which the membrane is operably attached. In this way, the half-well is inserted into the well.

In a further aspect, the plate defines an array of wells, and the divider includes a plurality of membranes. After the cells have been grown onto the membrane, the divider is inserted into the wells in a generally vertical orientation so that each well includes one of the membranes. In this way, the number of wells may be greatly increased, each having an appropriate cell layer to divide the wells into separate chambers. In one aspect, the divider comprises a plurality of teeth to which the membranes are operably attached. A bridge is provided to interconnect the teeth and may be grasped to insert the teeth into the array of wells. Alternatively, the divider may comprise an elongate member to which the membranes are operably attached.

In a further alternative, the divider comprises a plurality of half-wells which have a planar face to which the membranes are operably attached. Each of the dividers are configured so that they may be horizontally oriented to allow cells to be seeded on each of the membranes. The dividers may then be inserted into the wells in a vertical orientation.

The invention still further provides another exemplary method for performing assays. According to the method, a base member is provided having a plurality of wells. A top member is also provided and includes a plurality of apertures. In the method, cells are grown on to a membrane sheet which in turn is placed between the base member and the top member. The top member is secured to the base member such that at least some of the wells are aligned with at least some of the apertures. A substance is then introduced into the aligned apertures and is allowed to permeate the membrane sheet. A characteristic of the substance within the wells is then evaluated.

The evaluated characteristic can comprise the concentration of the substance. Based on the measured concentration, a permeation rate of the substance through the membrane sheet may then be determined. In one optional aspect, the wells may be filled with a buffer solution prior to placement of the membrane sheet between the top member and the base member. In another aspect, the substance may be maintained within a desired temperature range while permeating the membrane sheet. One particularly advantageous feature of the method is that the membrane sheet may be either horizontally oriented or vertically oriented during permeation. To facilitate evaluation of the characteristic, the membrane sheet may be removed from the base layer or may be punctured to gain access to the substance within the wells.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view of an exemplary testing device according to the invention.

Fig. 2 is a more detailed view of the device of Fig. 1 taken along lines 2-2.

Fig. 3 is a perspective view of a divider of the tester of Fig. 1 having a plurality of membranes for dividing wells into separate chambers.

Fig. 4 is a top perspective view of a strip of half-wells for dividing the wells of a multi-well plate into separate chambers when inserted therein according to the invention.

Fig. 5 is a bottom perspective view of the strip of half-wells of Fig. 4 showing a membrane attached to each half-well.

Fig. 6 is a top perspective view of an embodiment of a multi-well plate having an elongate slot extending through each row of wells according to the invention.

Fig. 7 is a top perspective view of a divider having a plurality of membranes which may be inserted into one of the slots of the plate of Fig. 6 according to the invention.

Fig. 8 is a top perspective view of the plate of Fig. 6 showing the divider of Fig. 7 being inserted into one of the slots according to the invention.

Fig. 9 is a top plan view of the plate of Fig. 8 having dividers inserted in each row.

Fig. 10 is a partial cross-sectional view of the plate of Fig. 9 taken along lines 10-10.

Fig. 11 is a perspective view of a further embodiment of a multi-well plate according to the invention.

Fig. 12 is a perspective view of a section of the plate of Fig. 11.

Fig. 13 is a perspective view of a divider for use with the plate of Fig. 11.

Fig. 14 is a schematic view of an exemplary system for performing permeation tests according to the invention.

Fig. 15 is a top view of another exemplary testing system according to the invention.

Fig. 16 is a exploded cross-sectional side view of the system of Fig. 15 taken along lines 16-16.

Fig. 17A is a perspective view of another exemplary testing system according to the invention.

Fig. 17B is an exploded view of the testing system of Fig. 17A.

Fig. 18 is an exploded cross-sectional side view of the system of Fig. 17A.

DETAILED DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The invention provides systems, devices and methods for testing and evaluating the transport or permeation of various substances through a layer of cells, and particularly epithelial cell layers, including those found in blood vessels throughout the body, arteries, the intestine, and the like. Although useful in a wide variety of simulation applications, the invention will find use in modelling intestinal permeation of drug-like compounds.

According to the invention, mammalian-based cells are seeded on a membrane while the membrane is in a generally horizontal orientation. Such an orientation is preferably employed to facilitate attachment of the mammalian-based cells to the membrane. In some embodiments, the membrane is then inserted into the wells of a multi-well plate, preferably in a generally vertical orientation, to divide the wells into separate chambers, such as a receptor chamber and a donor chamber. In some cases, however, it may be desirable to have the membrane inserted horizontally or at an incline. Vertical orientation of the membrane is advantageous in that it allows for a more efficient use of the space within the wells. In this way, a larger membrane surface may be provided to enhance the sensitivity of the system. Further, by optimizing the size of the membrane, smaller well sizes may be used while still providing an adequate transport interface.

In other embodiments, the membrane is inserted between a base member having a plurality of wells and a top member having a plurality of apertures, with at least some of the apertures corresponding to the wells of the base member. The assembly of the membrane between the base member and the top member forms top and bottom wells.

Once the membrane is inserted, various substances may be introduced into the top wells where they will permeate

through the cells on the membrane into the bottom wells. The permeation rate may be determined by measuring the concentration levels or other properties over time in both the top wells and the bottom wells. In this way, a scientist will be able to evaluate the permeation of various substances through the cells in order to model how the human body will absorb such substances through the intestinal wall.

The system of the invention will preferably employ a cell culture device which maintains cells in a tissue culture media until the cells are ready to be seeded onto the membrane. Preferably, the cells will be mammalian-based cells and the membrane will preferably be constructed of a porous material, such as a polytetrafluroethylene (commonly referred to as teflon), polyethylene, PCT, polycarbonate, or the like. For convenience of handling, the membranes will preferably be operably attached to a divider or surrounded by a raised edge or frame. In addition to facilitating handling of the membranes, the divider will preferably also encompass or frame the periphery of the membranes with a raised edge to constrain the growth of the cells to the membrane and to maintain rigidity of the membrane. In this way, growth of the mammalian-based cells onto the membrane will be facilitated. Use of such a divider is further advantageous in that it may be employed to horizontally orient the membranes when growing the cells on the membrane.

According to some embodiments, once a sufficient number of cells have been seeded onto the membranes, the divider may be removed from the cell culture device and placed into the wells of a multi-well plate. Exemplary plates which may be used with the invention comprise conventionally formatted multi-well plates, including 96-well plates, 384-well plates, and the like. Such multi-well plates may be "off the shelf" type plates or may be adapted to facilitate receipt of the divider depending on the particular configuration of the divider as described in greater detail hereinafter. Configuration of the membranes onto a removable divider as described herein is particularly advantageous in that the cells may more efficiently be seeded into the

membrane. Further, by vertically orienting the membranes in the wells, the surface area of the membranes is maximized to more effectively accommodate growth of mammalian-based cells. Moreover, such vertical orientation maximizes the transport interface to allow the membranes to be used within the relatively small wells of dense multi-well plates.

Either prior to or following insertion of the membranes into the wells, both the donor chambers (or top wells) and the receptor chambers (or bottom wells) will preferably be filled with a buffer solution. A variety of substances may then be introduced into the donor chambers where they will diffuse through the membranes and into the receptor chambers. Exemplary substances which may be introduced into the donor chambers include a wide variety of drug compounds, chemicals, and the like.

To facilitate introduction of such substances, the invention will preferably include a fluid delivery device, such as a pipette or multi-channel pipette. This may be done in an automated or manual manner. Following introduction of the substances into the donor chambers, various concentration measurements will be taken over time in both the donor chambers and receptor chambers to determine the permeation rate of the particular substance through the cells. The particular concentration may be evaluated using commercially available measuring equipment, such as an HPLC, a fluorescent plate reader, an absorbance plate reader, and the like.

Referring now to Figs. 1-3, an exemplary embodiment of a testing device 10 will be described. Device 10 comprises a plate 12 having a plurality of wells 14. Each well 14 includes an open top end and a pair of grooves 16 which are vertically oriented when plate 12 rests upon a horizontal surface. As shown, plate 12 includes 96 wells. However, it will be appreciated that plate 12 may be provided with larger or smaller numbers of wells. Further, wells 14 may be organized into a two-dimensional array as shown or in other configurations.

Testing device 10 further comprises a divider 18 having a bridge 20 and a plurality of teeth 22. As best shown

in Figs. 2 and 3, each tooth 22 defines a central opening to which a membrane 24 is attached. With this configuration, divider 18 may be inserted into a row of the wells as shown, with teeth 22 sliding within grooves 16 to vertically orient membranes 24 within wells 16 and to divide each well into a receptor chamber 26 and a donor chamber 28. Hence, divider 18 is advantageous in that it may be removed from testing device 10 so that cells may be grown on membranes 24 while the membranes are in a horizontal orientation. After a layer of cells has grown on membranes 24, divider 18 may be inserted into a row of wells 14 to divide the wells and to place the membranes in a vertical orientation. A substance, such as a chemical or drug, may then be placed into donor chamber 28 where it will diffuse through membrane 24 and into receptor chamber 26. Concentration measurements may then be taken in both the receptor chamber 26 and donor chamber 28 to determine the permeation rate of the substance through the cells on membrane 24.

The surface area of membrane 24 may vary depending upon a variety of factors, including the size of wells 14, the type of cells being used, the analytical techniques available to measure the amount of material transported, and the like. The surface area should be large enough so that it will adequately model a desired cell layer. To assist in growing the cells on membrane 24, teeth 22 will preferably define a raised edge which encompasses the periphery of membrane 24. Exemplary materials for constructing membrane 24 comprise polytetrafluroethylene, polyethylene, PET, polycarbonate, and the like.

Plate 12 will preferably have overall dimensions which are similar to those used with commercially available 96-well plates so that plate 12 may be used with commercially available processing equipment. However, it will be appreciated that as denser plates become more commercially accepted, divider 18 may be modified to accommodate more teeth and membranes so as to be useful with denser plate designs.

Referring to Figs. 4 and 5, an alternative embodiment of a divider 30 will be described. Divider 30

comprises a plurality of half-wells 32 which are connected by a strip 34. Half-wells 32 include a planar face 36 which each define an opening over which a membrane 38 is attached. Face 36 preferably defines a raised edge around membranes 38 similar to divider 18 as previously described.

Divider 30 is configured to be inserted into the wells of a conventional multi-plate well. As shown, divider 30 includes eight half-wells which may be inserted into a row of wells of a conventional 96-well plate. Alternatively, fewer or more half-wells may be provided for insertion into different configurations of multi-well plates. When divider 30 is inserted into a row of wells, each well is divided into a donor chamber 40 (defined by the half-well) and a receptor chamber (not shown). In this way, various substances may be diffused through membranes 38 to determine the absorption by the cells on the membrane similar to the embodiment previously described in connection with Fig. 1. Likewise, divider 30 is removable from the multi-well plate so that cells may be grown on membranes 38 while in a generally horizontal orientation. When inserted into wells of a multi-well plate, the half-well design will orient membranes 38 in a generally vertical orientation.

Referring now to Figs. 6 and 7, an alternative testing device will be described which comprises a multi-well plate 42 (see Fig. 6) and a divider 44 having a plurality of membranes 46 (see Fig. 7). Plate 42 includes a plurality of wells 48 which are similar to wells of commercially available multi-well plates (only two rows of wells are illustrated for purposes of convenience). Extending between each row of wells is an elongate slot 50. Pins 52 are provided at each end of slot 50 to assist in sliding dividers 44 into the correct location so that the membranes are correctly positioned in the wells without damaging the membranes. Slot 50 is adapted to receive divider 44 so that each membrane 46 divides each well 48 into a receptor chamber and a donor chamber similar to the embodiments previously described. As with other embodiments, divider 44 will preferably define raised edges around membranes 46 to constrain cell growth to the membranes.

Insertion of divider 44 into slots 50 is best illustrated in Figs. 8-10. As shown, each row of wells receives a separate divider 44 so that each well 48 will be divided into a receptor chamber and a donor chamber. As shown in Fig. 10, slot 50 extends below the bottom surface of each well 48 to prevent fluids from leaking around divider 44. Further, divider 44 will preferably be configured so that a seal will be created between divider 44 and plate 42 when divider 44 is inserted into slot 50. In this way, liquids will be prevented from seeping into adjacent wells.

A modification of plate 42 is illustrated in Fig. 11 and is identified by reference number 70. Plate 70 is formed of a plurality of sections 72 which are placed together to form a plurality of wells 74. As best shown in Fig. 12, section 72 includes two rows of half-wells which are arranged so that when the sections are positioned adjacent each other, wells 74 will be formed as shown in Fig. 11. A divider 76 which is similar to the other dividers as previously described is positioned between two of the sections 72 as shown in Fig. 11 to divide the wells into separate chambers in a manner similar to that described with other embodiments. As best shown in Fig. 13, divider 76 includes a plurality of membranes 78 to which cells may be grown similar to other embodiments described herein.

One advantage of plate 70 is that it may be placed in a housing 80 (see Fig. 11) which services to align sections 72 and dividers 76 in their p roper arrangement. Further, once in place, a clamp or other securing device (not shown) is employed to squeeze or force the sections 72 together. In this way, a tight seal will be provided between the sections and the dividers to prevent liquids from leaking from the wells. Hence, with this configuration, permeation tests may be performed without having the liquids leaking from the wells.

Referring now to Fig. 14, an exemplary system 54 for testing the permeation or transport of a substance across a cell layer will be described. System 54 includes a cell culture device 56 which is employed to produce monolayers of

epithelial cells, such as Caco-2 (Colon Carcinoma) or MDCK (Canine Kidney). Such cells in turn are used with the present invention as a model to study passive drug absorption across the intestinal epithelium in vitro. The confluent monolayers are subcultured whenever required by treatment with trypsin, EDTA and Phosphate-buffered saline (PBS). These cultures are maintained in a sterile environment within device 56 and incubated at 37 degrees C in a humidified atmosphere at 5% CO_2, 95% air.

When the cells are ready to be placed onto the membrane, the membrane is arranged in a horizontal orientation. The trypsinized cells are then seeded as epithelial layers onto the membrane. Any of the membrane configurations previously described herein may be used to receive the cells.

System 54 further includes a multi-well plate 58 which may be configured to be similar to any of the multi-well plates previously described herein. Multi-well plate 58 is configured to receive the membranes after the cells from cell culture device 56 have been seeded onto the membranes. The membranes are preferably arranged in a vertical orientation (although other orientations may be used) within plate 58 to separate the wells into a donor chamber and a receptor chamber as previously described. System 54 further includes a chemical source 60 which contains a chemical that is to be tested within plate 58. Exemplary chemicals which may be tested include a wide variety of drug compounds which would require transport across epithelial cells.

To deliver the chemicals from source 60 to the wells of plate 58, a fluid delivery device 62 is provided. Fluid delivery device 62 may comprise, for example, a single pipette, a multi-channel pipette, or an automated multi-channel pipetting system that takes chemicals from source 60 and places them into the donor chambers of plate 58. To facilitate the testing procedure, each of the wells may be provided with a buffer solution into which the chemical is placed.

After the chemical has had a chance to diffuse into the receptor chamber, concentration measurements for the chemical are taken in both the donor chamber and the receptor chamber with a concentration measuring device 64. Such a concentration measuring device may comprise, for example, an HPLC, a fluorescent plate reader, an absorbance plate reader, and the like. The concentration measurements will preferably be taken over time so that the permeation rate of the chemical through the cell layer may be determined.

Referring now to Figs. 15-16, an exemplary embodiment of a testing system 100 for measuring the apparent permeability values of compounds through monolayers of cells that are immobilized on a membrane in a high throughput manner will be described. System 100 comprises a base member 102, a top member 104 and a membrane sheet 106. Membrane sheet 106 includes at least one layer of cells 18 which are preferably grown on membrane sheet 106 prior to being placed between base member 102 and top member 104.

Base member 102 includes a plurality of wells 110 which are aligned with a plurality of apertures 112 within top member 104 when top member 104 is aligned with and secured to base member 102.

Optionally provided is a gasket 114 having a plurality of apertures 116 which correspond to apertures 112 of top member 104 to assist in providing an appropriate seal between top member 104 and the membrane sheet 106 or the base member 102 and the membrane sheet 106 when secured together. A variety of securing mechanisms and devices may be employed to secure top member 104 to base member 102, such as, for example, clamps, screws, and the like. For instance, as illustrated in Fig. 1, a plurality of screws 118 extend through top member 104 to hold top member 104 to base member 102. Top member 104 and based member 102 will preferably include flat surfaces so that an adequate seal will be provided to prevent liquids from leaking from apertures 112 or wells 110.

Membrane sheet 106 will preferably constructed of a porous material, such as polycarbonate, PTFE, and the like.

Membrane sheet 106 is removable from the system so as to allow cells to be grown onto the sheet to confluency, preferably as determined by their transepithelial electrical resistance (TEER) or impermeability to appropriate marker compounds (e.g., lucifer yellow).

Base member 102 will preferably include 96 wells which are arranged in a conventional format so that system 100 may be used with standard tools designed for use with 96 well microtiter dishes. For example, by arranging system 100 in this manner it may be readily integrated into a robotocized platform which performs compound addition, sampling, and analysis manipulations. However, it will appreciated that other numbers of wells and format types may be employed within the principles of the present invention.

In use, cells are preferably grown onto membrane sheet 106 as previously described in this application. Wells 110 of base member 102 are then preferably filled with an appropriate transport buffer solution. When system 100 is used in a horizontal orientation, each of wells 110 will preferably be filled to capacity. If used in a vertical orientation, wells 110 may be filled to less than capacity.

Membrane sheet 106 containing cells 108 is then inserted between base member 102 and top member 104 as illustrated in Fig. 16. Members 102 and 104 are then secured together so as to form a junction to prevent liquids from leaking around membrane 106. When appropriately secured together, apertures 112 are filled with a buffer containing an appropriate test compound or compounds.

For testing in the horizontal orientation, system 100 is placed at an appropriate temperature for a desired length of time. Optionally, system 100 may be shaken during the testing procedure. The solution within apertures 112 is then removed and each aperture 112 is washed and aspirated to remove any residual compound. The solutions within cells 110 are then evaluated to determine the concentration of the test compound that has permeated through membrane sheet 106. Access to wells 110 may be accomplished by puncturing membrane sheet 106, e.g., with a pipette, or by disassembling system

100. For testing in the vertical orientation, a cover will preferably be placed over apertures 112 to prevent spillage of their contents. Testing then proceeds as previously described for the horizontal orientation.

Based on the concentration of the substance within wells 110, the apparent permeability of the compounds through cells 108 may be determined. By providing a large number of wells, system 100 facilitates the ability to conduct permeation studies with multiple compounds simultaneously. Further, when the wells are arranged in a standard format, standard tools designed for use with 96 well microtiter plates may be employed. Arrangement of wells 110 is further advantageous in that testing may occur with considerably less compounds than has been required with other approaches. Further, by constructing membrane sheet 106 to be removable, the cell monolayers may be prepared in an efficient manner to further facilitate the testing procedure.

While system 100 has been illustrated in a 96 well format, it will be appreciated that a variety of well shapes, geometries, sizes, and the like may be employed within the principles of the invention. System 100 may optionally contain electrodes for determining TEER (Trans-Epithelial Electrical Resistance) values. Further, sampling portions may be provided to facilitate sample addition and removal. Temperature control capabilities could also be provided to monitor and control the temperature during the testing procedure.

Referring now to Figs. 17A, 17B and 18, a preferred embodiment of a testing system 120 for measuring the permeability of compounds through cell monolayers will be described. System 120, similar to system 100 described above, comprises a base member 122 having a plurality of wells 123, a top member 124 and a membrane sheet 126. Membrane sheet 126 includes a layer of cells 128 which are preferably grown on membrane sheet 126 prior to its being placed between base member 102 and top member 124.

Top member 124 comprises a cylindrical body 130 having an upper face 132 and a lower face 133, and may

optionally be used with a gasket 134. Top member 124 further includes an array of apertures 136 spaced to correspond to and align with wells 123 in the base member. Each aperture extends from upper face 132 to lower face 133 to form a plurality of bottom openings 138 in lower face 133. Each of bottom openings 138 defines an aperture perimeter. Gasket 134 also include openings 139 which preferably correspond in size and shape to openings 138.

Base member 122 includes a top face 140. Wells 123 of base member 122 each have a top end 142 defining a well perimeter in top face 140, and a bottom end 144.

Referring now to Fig. 18, for each corresponding aperture and well, the aperture perimeter (defined by openings 138 in top assembly 124) is greater than the well perimeter (at top ends 142 in base member 122). This may be accomplished, for example, by bevelling the edges of openings 138. This feature results in a more reliable seal between top member 124 and base member 122. Specifically, when layer of cells 128 is contacted with either gasket 134 or lower face 133 of top member 124, cells adjacent the aperture perimeter tend to get damaged, disrupting the integrity of the cell monolayer in that region. If the aperture perimeter is identical to or smaller than the well perimeter, such a disruption in monolayer effectively short-circuits the monolayer system, rendering that well unreliable or ineffective at measuring permeability across the monolayer.

According to the present invention, however, effects of disruptions of the cell monolayer by an overlying top member 124 are minimized by isolating such disruptions to regions outside the well perimeter. This, in combination with the natural seal that forms between the bottom of membrane 126 and base member 122, limits the "short circuiting" effects of such disruptions to a level where experiments can be conducted reliably and consistently.

In a preferred embodiment, membrane 126 is mounted in a rigid support or frame 150, facilitating transfer of the membrane to and from the test device and providing a degree of rigidity to membrane 126. Preferably, frame 150 comprises a

ring or cup-shaped member as illustrated in Figs. 17A and 17B. Such a membrane on a rigid support can be obtained, e.g., from Costar as Cat #3419. However, it will be appreciated that other types and geometric configurations of frames may be provided to hold the membrane, including square, rectangular, triangular, oval, and the like.

Use of a cup-shaped frame is particularly advantageous in that such frames and membranes are commercially available as just described. In this way, known techniques may be employed to efficiently culture cells onto membrane 126. Once cultured, the membrane may be used to produce multiple wells within system 120 in a manner similar to system 100. In this way, the efficiency of the testing procedure is greatly increased. Further, the wells in system 120 are preferably arranged in a standard format, such as an 96 well array, so that system 120 may be used with standard tools similar to system 100.

As best shown in Figs. 17A and 17B, frame 150 includes a lip 152. Base member 122 also includes a wall 154 having a groove or a guide 156. To position membrane 126 on base member 124, frame 150 is moved over face 140 until lip 152 is received into guide 156. Lip 152 slides within guide 154 until fully inserted as shown in Fig. 17A. In this way, wall 154 serves both to position frame 150 and membrane 126 at a known position relative to face 140 and to secure membrane 126 over face 140. Further, such a manner of insertion significantly reduces the chances for damaging the cells on membrane 126.

Once frame 150 is fully inserted, top member 124 is inserted into frame 150 until it rests on membrane 126. As best shown in Fig. 17B, top member includes a pair of alignment pins 157 (one being hidden from view) which protrude from lower face 133, and base member 122 includes a pair of corresponding holes 159 (one being hidden from view) in top face 140. Pins 157 and holes 159 serve to align top member 124 with base member 122 and to prevent rotation of the two members relative to each other to prevent damage to the cells on membrane 126.

Because frame 150 is at a known location relative to face 140, top member 124 is also at a known position relative to face 140. In this manner, a way is provided to reproducibly allow apertures 136 in top member to be aligned with wells 123 in base member each time the system is assembled. After assembly, screws (not shown) are screwed into holes 158 of top member 124 and into holes 160 of base member to secure membrane 126 between top member 124 and base member 122. However, it will be appreciated that other securing devices may be used, including clamps, latches, and the like.

One particular advantage of system 120 is that multiple base members may be provided, with each base member having wells which define a different volume. In this way, the flow rate of substances into the wells may be controlled simply by selecting a base member with the appropriately sized wells.

System 120 may be operated in a manner similar to system 100 to perform permeations tests. Once the experiment is finished, samples may be withdrawn from apertures 136. Apertures 136 are then washed with a solvent one or more times. Membrane 126 may then be removed or punctured to access wells 123 to allow the solution to be extracted. As previously described either manual or automated equipment may be employed to add and withdraw the various fluids.

The invention has now been described in detail. However, it will be appreciated that certain changes and modifications may be made. Therefore, the scope and content of this invention are not limited by the foregoing description. Rather, the scope and content are to be defined by the following claims.

WHAT IS CLAIMED IS:

1. A testing system, comprising:

a base member having a plurality of wells;

a top member having a plurality of apertures, at least some of which correspond to the wells in the base member; and

a membrane sheet coupled to a frame, wherein the membrane sheet is adapted to receive a layer of cells and to be placed between the top member and the base member, wherein a substance within the apertures may permeate through the membrane and into the wells.

2. A system as in claim 1, further comprising a gasket which is adapted to be placed between the base member and the top member, and a securing mechanism to secure the top member to the base member.

3. A system as in claim 1, wherein the membrane is constructed of a material selected from the group of materials consisting of polytetrafluroethylene, polyethylene, PET and polycarbonate, and wherein the cells comprise mammalian based cells.

4. A system as in claim 1, wherein the frame comprises a geometrically-shaped member disposed around a periphery of the membrane, and wherein the base member includes a guide to receive at least a portion of the frame to allow the membrane to be positioned on the base member at a known location.

5. A system as in claim 4, wherein the top member is received into the frame to assist in aligning the apertures with the wells.

6. A system as in claim 1, wherein the wells and apertures are arranged in a two dimensional array.

7. A system as in claim 1, further comprising a second base member having wells with a different volume than the wells of the first base member.

8. A method for performing assays, comprising:

providing a base member having a plurality of wells, and a top member having a plurality of apertures;

growing cells onto a membrane sheet that is coupled to a frame;

grasping the frame and positioning the membrane sheet onto the base member;

placing the top member over the membrane;

securing the top member to the base member such that at least some of the wells are aligned with at least some of the apertures;

introducing a substance into the aligned apertures and allowing the substance to permeate the membrane sheet; and

evaluating a characteristic of the substance within the wells.

9. A method as in claim 8, wherein the characteristic comprises the concentration of the substance, and further comprising determining a permeation rate of the substance through the membrane sheet.

10. A method as in claim 8, further comprising filling the wells with a buffer solution prior to placement of the membrane sheet between the top member and the base member.

11. A method as in claim 8, further comprising maintaining the substance within a desired temperature range.

12. A method as in claim 8, further comprising horizontally orienting the membrane sheet during permeation.

13. A method as in claim 8, further comprising vertically orienting the membrane sheet during permeation.

14. A method as in claim 8, further comprising removing the membrane sheet from the base layer or puncturing the membrane sheet to evaluate the characteristic.

15. A method as in claim 8, wherein the membrane is coupled to a frame, and further comprising inserting at least a portion of the frame into a guide of the base member and inserting the top member into the frame until the top member rests on the membrane.

16. A testing system, comprising:

a base member having a first face and a plurality of wells, each well having a top end defining a well perimeter in the first face, and a bottom end;

a top member having second and third faces and an array of apertures spaced to correspond to the wells in the base member, wherein each aperture extends from the second face to the third face to define an aperture perimeter in the third face; and

a membrane sheet adapted to receive a layer of cells and to be placed between the top member and the base member, wherein for each corresponding aperture and well, the aperture perimeter is greater than the well perimeter.

17. A system as in claim 16, further comprising a gasket disposed against the third face, and wherein the gasket has a plurality of openings which are aligned with the aperture perimeters, and further comprising a securing mechanism to secure the top member to the base member.

18. A system as in claim 16, wherein the membrane sheet is constructed of a material selected from the group of materials consisting of polytetrafluroethylene, polyethylene, PET and polycarbonate.

19. A system as in claim 16, further comprising a frame coupled to the membrane, wherein the frame comprises a

geometrically-shaped member disposed around a periphery of the membrane.

20. A system as in claim 19, wherein the base member includes a guide to receive at least a portion of the frame to allow the membrane to be positioned on the base member at a known location, and wherein the top member is received into the frame to assist in aligning the apertures with the wells.

21. An apparatus for testing permeability of a sheet of cells on one side of a membrane, comprising;

a base member having a plurality of wells, each well having a top end and a bottom end;

a top member having a plurality of apertures, each having a top opening and a bottom opening, said bottom openings each defining a beveled edge, wherein said apertures are spaced to correspond to the wells; and

a clamping mechanism to secure said membrane between said top and base members.

22. A system as in claim 21, further comprising a frame coupled to the membrane, and wherein the frame comprises a geometrically-shaped member disposed around a periphery of the membrane.

23. A testing device comprising:

a plate defining at least one well; and

at least one membrane which is insertable into the well to allow cells to be grown onto the membrane prior to insertion into the well.

24. A device as in claim 23, wherein the membrane is insertable into an open top end of the well in a generally vertical orientation, and wherein the cells comprise mammalian-based cells.

25. A device as in claim 1, further comprising a divider to which the membrane is operably attached, the divider being insertable into the well to hold the membrane in a generally vertical orientation within the well.

26. A device as in claim 25, wherein the divider encompasses a periphery of the membrane, and wherein the divider forms a raised edge around the periphery of the membrane to constrain cell growth to the membrane.

27. A device as in claim 25, wherein the well includes at least one generally vertically oriented slot into which the divider is received.

28. A device as in claim 25, wherein the plate defines an array of wells, and further comprising a plurality of membranes which are insertable into the wells in a generally vertical orientation.

29. A device as in claim 25, wherein the divider comprises a plurality of teeth to which the membranes are operably attached and a bridge interconnecting the teeth, wherein the teeth are insertable into the array of wells to place a separate membrane into each well.

30. A device as in claim 25, wherein the divider comprises an elongate member to which the membranes are operably attached, wherein the plate includes an elongate slot extending between the array of wells, and wherein the elongate member is insertable into the slot to place the membranes into the wells.

31. A device as in claim 25, wherein the array is a two-dimensional array, with the wells being arranged in rows and columns, and further comprising a plurality of row dividers, each having a plurality of membranes operably attached thereto for insertion into each row of wells.

32. A method for performing assays, the method comprising:

placing a membrane into a well to divide the well into a donor chamber and a receptor chamber;
growing cells onto the membrane; and

introducing at least one substance into the donor chamber and allowing at least some of the substance to diffuse through the membrane and into the receptor chamber; and

evaluating a characteristic of the substance that is within the donor chamber and the receptor chamber.

33. A method as in claim 31, wherein the evaluated characteristic comprises the concentration of the substance.

34. A method as in claim 31, wherein the evaluated characteristic comprises determining the permeation rate of the substance across the membrane, and further comprising determining the permeation rate of the substance through the cells on the membrane based at least in part on the concentration of the substance within the donor chamber and the receptor chamber over time.

35. A method as in claim 31, wherein the substance comprises a chemical, and further comprising introducing a buffer solution into the chambers prior to placing the chemical into the donor chamber.

36. A method as in claim 31, further comprising seeding the cells onto the membrane while the membrane is in a generally horizontal orientation, and placing the membrane into the well in a generally vertical orientation, and further comprising growing the cells onto the membrane prior to placement into the well.

37. A method as in claim 31, further comprising constraining the growth of the cells onto the membrane.

38. A method as in claim 31, further comprising a divider to which the membrane is operably attached, and further comprising inserting the divider into the well in a generally vertical orientation.

39. A method as in claim 37, wherein the well includes at least one generally vertically oriented slot, and further comprising sliding the divider through the slot when inserting the divider into the well.

40. A method as in claim 37, wherein the plate defines an array of wells, wherein the divider includes a plurality of membranes, and further comprising inserting the divider into the wells in a generally vertical orientation so that each well includes one of the membranes.

41. A method as in claim 37, wherein the divider comprises a plurality of teeth to which the membranes are operably attached and a bridge interconnecting the teeth, and further comprising inserting the teeth into the array of wells to place a separate membrane into each well.

42. A method as in claim 37, wherein the divider comprises an elongate member to which the membranes are operably attached, wherein the plate includes an elongate slot extending between the array of wells, and further comprising inserting the elongate member into the slot to place the membranes into the wells.

43. A method as in claim 37, wherein the array is a two-dimensional array, with the wells being arranged in rows and columns, further comprising a plurality of row dividers, each having a plurality of membranes operably attached thereto, and further comprising inserting one divider into each row of wells.

44. A testing system comprising:

a plate comprising a plurality of individual sections which are arranged to form a plurality of wells; and

at least one membrane which is received between two of the sections so as to divide at least one of the wells into separate chambers.

45. A system as in claim 43, wherein the membrane is received between the sections so as to be generally vertically oriented.

46. A system as in claim 43, further comprising a base which holds the individual sections, and a clamp to hold the sections together within the base.

47. A system as in claim 43, further comprising a plurality of membranes which are spaced apart such that each well is divided into separate chambers by the membranes.

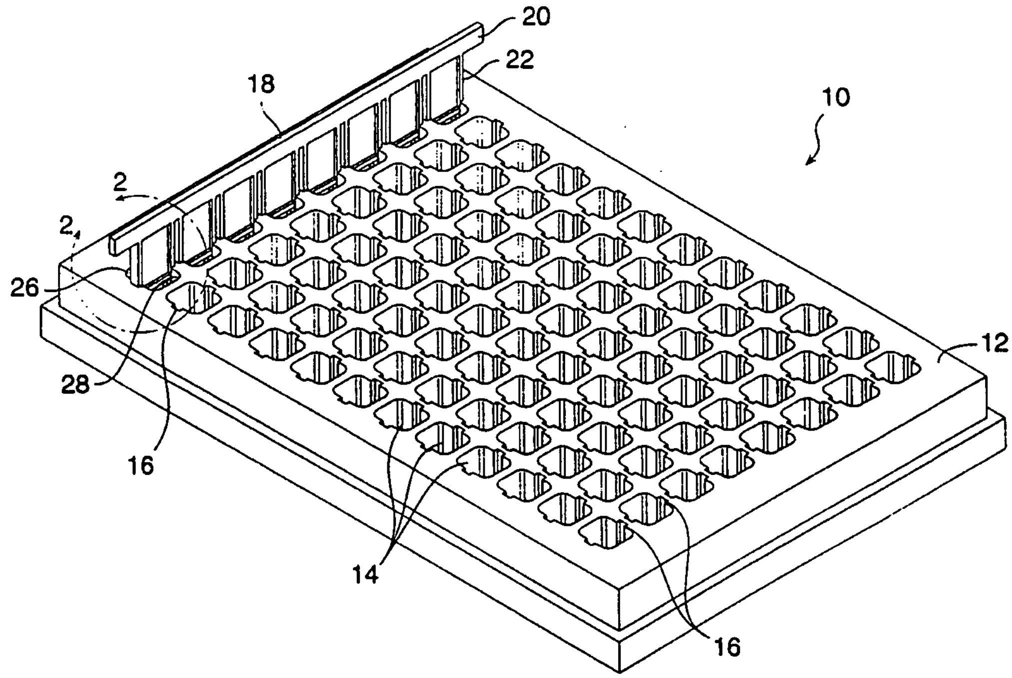

FIG. 1

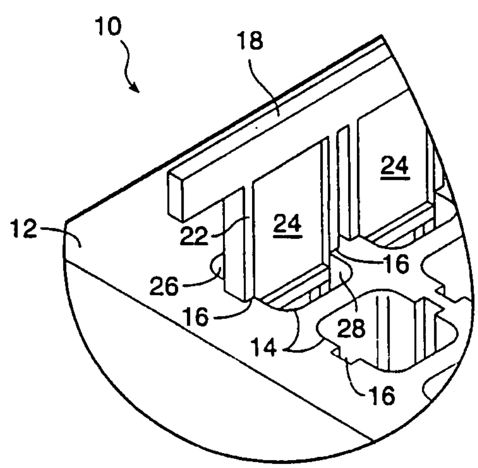

FIG. 2

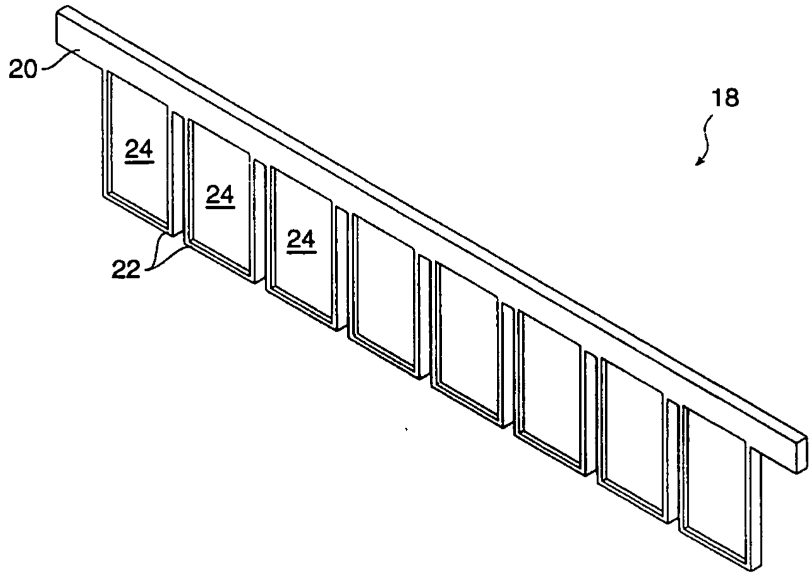

FIG. 3

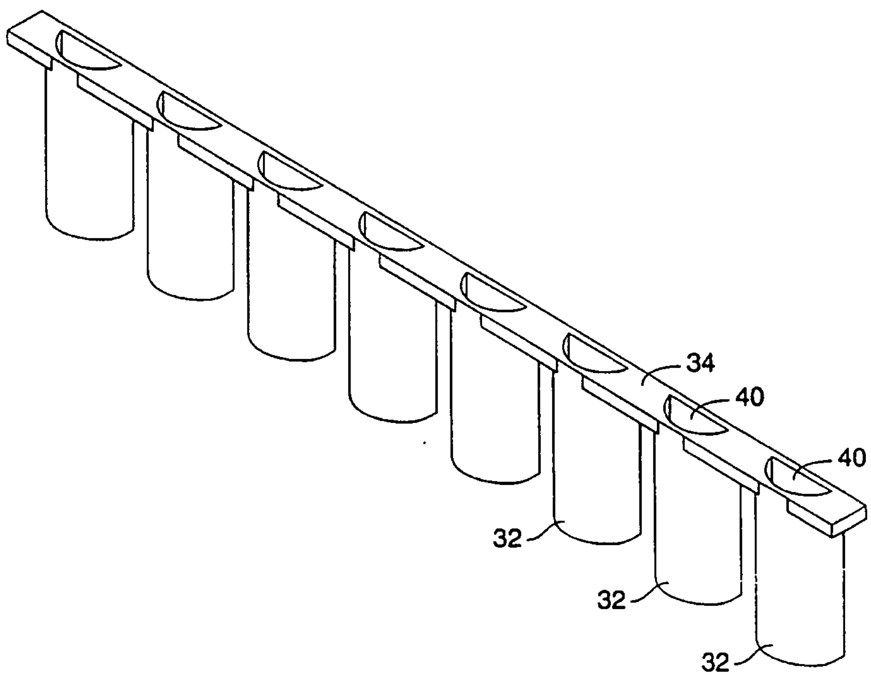

FIG. 4

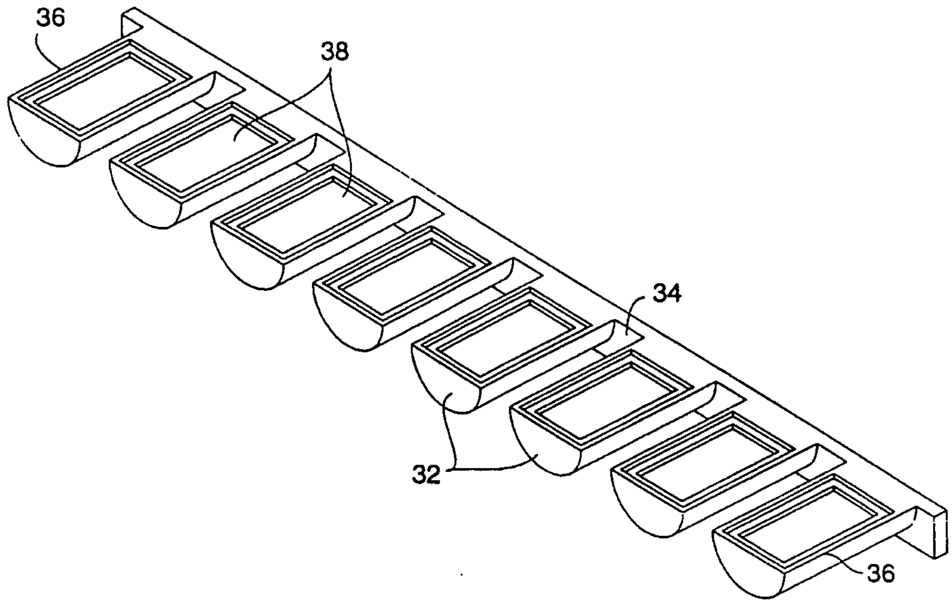

FIG. 5

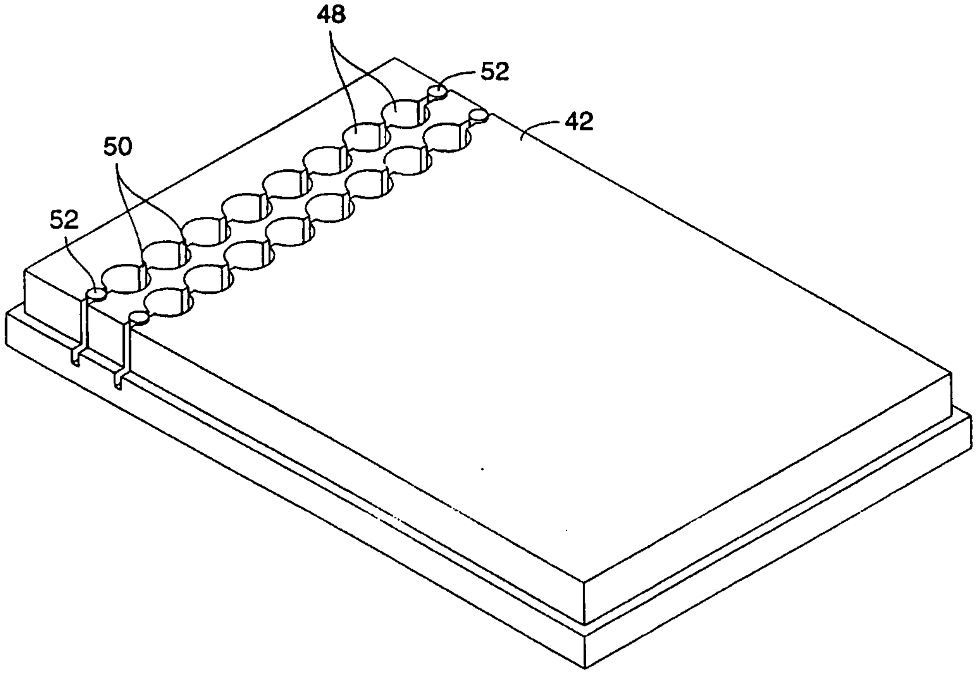

FIG. 6